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THE INFLUENCE OF TWO STARTER CULTURES ON THE MICROBIOLOGICAL STABILITY OF *MACEDONIAN TRADITIONAL SAUSAGE*

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Abstract: The aim of this paper is to monitor the influence of two starter cultures on the microbiological stability of *Macedonian traditional sausage*. The research covered three variants: Control variant; Variant 2: with addition of starter culture CS-300; Variant 3: with addition of starter cultures CS-300 and BLC-78. The total bacteria count and *Lactobacillus* sp. in all three variants decreases compared to the initial value. There is no presence of *Escherichia coli* and *Enterobacteriaceae*.Starter culture CS-300 is recommended, while better stability of the microflora is achieved during the storage period, as well as a good quality. At the same time, the use of nitrite salt is eliminated, which results in getting a safe product.

Keywords:traditional sausages, starter cultures, microorganisms

Introduction

Sausages are products obtained by filling of natural or artificial wrappers with a mixture of different types and quantities of minced meat, fatty tissues, cuticles, internal organs, connective tissue residues and additional ingredients. Today, sausages belong to the largest group of meat products available in a wide range of types and with different commercial names (Beuchat and Montville, 2001).

The presence of microorganisms in meat, as an inevitable factor for the production of various types of meat products, is a basic impetus for research into the composition and action of native microflora(Ђукићand Мандић, 2012), which is the basis for developing the use of starter cultures in the meat industry. But despite all the positive properties in terms of quality of the final product, sometimes the development of undesirable microflora can occur which causes various forms of spoilage of the product (Ђукић et al., 2015). In the United States, bacteria of the genus *Lactobacillus* sp. and *Pediococcus* sp. are most commonly used as starter cultures, while in Europe, in addition to these bacteria, *Micrococcus* sp. and *Staphylococcus* sp. are also used in the meat industry. The application of starter cultures enables a dominant growth of the desired microflora in relation to the contaminant microflora that may be present in fresh meat or in the raw materials that are added during the production of sausages (Vidal and Talon,2007). In addition to the dominant desirable microflora, certain undesirable or

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pathogenic species of microorganisms sometimes develop in meat products, as a result of the creation of favourable conditions for them (Honikel, 2008). Meat products are a medium that is very suitable for the growth and development of many types of microorganisms (Fontana et al., 2005). On the other hand, sausages are a product made from minced meat due to which microorganisms have the opportunity to spread faster throughout the product (Aymerich et al., 2003).The use of certain types of microorganisms or their metabolic products is the basis for the development of new technologies, which can contribute to the standardization of the production process and achieve uniform quality (Chartier and Couture, 2004). So, the use of starter cultures in the food industry is of great economic importance, which contributes to reducing or eliminating the problems that occur in the quality and safety of food products, but also can influence to the global food deficit.

The aim of this paper is to monitor the influence of two starter cultures on the microbiological stability of industrially produced *Macedonian traditional sausage*.

Material and methods

As a material for work was used *Macedonian traditional sausage* produced in industrial conditions in the meat industry "Soleta" in Skopje. As a basis the traditional formulation of *Vevcanski* sausage, modified for industrial use. Pork meat (I category) and dorsal bacon were used in the ratio 75:25%. Water was added in an amount of 150g/kg mixture. Then additives, spices and starter cultures were added to the mixture.The following starter cultures were used: CS-300 *(Staphylococcus carnosus* ssp. *utilis*) and BLC-78 (*Pediococcus acidilactici + Staphylococcus carnosus*).

The research covered three variants:*Variant 1*: Control variant (with using of nitrite salt);*Variant 2*: The basic formulation was enriched by the addition of starter culture CS-300 in combination with powder Swiss chard and powdered acerola;*Variant 3*: The basic formulation was enriched by the addition of starter cultures CS-300 and BLC-78 in combination with powder Swiss chard and powdered acerola. The aim of adding Swiss chard powder is to provide a natural source of nitrates that the added starter cultures will convert into nitrites with which it is expected to achieve better results compared to the control variant where nitrite salt is added during production, and the only source nitrates is the leek that is part of the basic formulation. In this way, not only nitrite salt is completely excluded from use, thus eliminating its adverse effects on the health of consumers, but also improvements in the quality of sausages have been achieved. The meat, together with the added spices and starter cultures, was mechanically mixed in a stirrer. Then it was accessed to machine filling of the sausages. Sausages were then thermally treated according to a program that was created according to the needs and modification of the basic formulation.

Then, a total bacteria count, *Lactobacillus* sp., as well as the presence of *Escherichia coli* and *Enterobacteriaceae* were determined in the raw material

(fresh meat), in the filling (fresh meat with spices and starter cultures) and in the final product (sausages) on the 10th, 20th and 30th day of the production, in a total of six samples taken at random from each variant separately. The determination of the total number of aerobic mesophilic bacteria was performed according to the method of serial dilution of MPA (mesopeptone) agar, and the bacteria of the genus *Lactobacillus* sp. on MRS (De Man, Rogosa and Sharpe) agar. The incubation period was 72 hours at 30°C.*Enterobacteriaceae* and *Escherichia coli* were determined of VRBG (Violet Red Bile Glucose) agar, i.e. ECC ChromoSelect Selective agar respectively. The incubation period was 24 hours at 37°C.The analysis was performed according to ISO 7218.

Results and discussion

According to the data presented in Table 1, can be seen that the total bacteria count in the fresh meat used for the production of *Macedonian traditional sausages* is 6.1×10^5cfu/g. The number of *Lactobacillus*sp. in the fresh meat is 7.0×10^3cfu/g.The total bacteria count as well as the *Lactobacillus* sp. has slightly higher values in the filling compared to the raw material (Table 1). This is due, above all, to the microflora present in the added ingredients, the manual activities for obtaining the necessary mixture for filling the sausages and the added starter cultures (in the variants 2 and 3).

Tabela 1.Mikrobiološkaanalizasvežeg mesa i punjenja(cfu/g)
Table 1. Microbiological analysis of the fresh meat and filling(cfu/g)

Varijanta *Variant*	n	Ukupanbrojbakterija *Total bacteria count*	*Lactobacillus* sp.	*Escherichia coli*	*Enterobacteriaceae*
		$\bar{x}$± SD	$\bar{x}$± SD	$\bar{x}$± SD	$\bar{x}$± SD
Svezemeso *Fresh meat*	3	6.1×10^5	7.0×10^3	/	/
Varijanta 1 *Variant 1*	3	$7.2^a\times 10^5$ ± 21.21	$7.7^a\times 10^4$ ± 7.07	/	/
Varijanta 2 *Variant 2*	3	$9.3^b\times 10^5$ ± 10.61	$9.6^b\times 10^4$ ± 0.71	/	/
Varijanta 3 *Variant 3*	3	$9.6^c\times 10^5$ ± 14.14	$9.9^c\times 10^4$ ± 10.61	/	/

^{a,b,c}– the values marked with different letters have a statistically significant difference between the examined variants($p<0.05$)

The total bacteria count determined in the filling in variants 2 and 3 is statistically significant different ($p<0.05$) compared to the control variant. This difference is primarily due to the added starter cultures in the filling of variants 2 and 3 (Zdolec et al., 2007; Kimiran et al., 2014). The increase in the number of bacteria occurs in the incubation phase of the added starter cultures (Zara et al., 2007). The dynamics of the number of *Lactobacillus* sp. is followed the dynamics of

the total bacteria count (Janssens et al., 2012). The number of *Lactobacillus* sp. in variants 2 and 3 showed a statistically significant difference ($p<0.05$) compared to the control variant. Nevertheless, it can be seen that there is no presence, neither of *Escherichia coli* nor of *Enterobacteriaceae*.

Tabela 2.Mikrobiološkaanalizakobasica(cfu/g)
Table 2. Microbiological analysis of the sausages (cfu/g)

Parametar *Parameter*	n	Varijanta 1 *Variant 1*	Varijanta 2 *Variant 2*	Varijanta 3 *Variant 3*
		$\bar{x}$± SD	$\bar{x}$± SD	$\bar{x}$± SD
10 danproizvodnja *10th day of the production*				
Ukupanbrojbakterija *Total bacteria count*	3	$5.7^{a}\times 10^{5}\pm 13.43$	$7.1^{b}\times 10^{5}\pm 10.60$	$7.7^{c}\times 10^{5}\pm 10.60$
Lactobacillus sp.	3	$7.1^{a}\times 10^{4}\pm 10.61$	$8.9^{b}\times 10^{4}\pm 14.14$	$9.0^{c}\times 10^{4}\pm 9.19$
Escherichia coli	3	/	/	/
Enterobacteriaceae	3	/	/	/
20 danproizvodnja *20th day of the production*				
Ukupanbrojbakterija *Total bacteria count*	3	$4.8^{a}\times 10^{4}\pm 7.07$	$4.5^{b}\times 10^{4}\pm 7.77$	$4.9^{c}\times 10^{4}\pm 7.07$
Lactobacillus sp.	3	$9.1^{a}\times 10^{3}\pm 7.07$	$9.8^{b}\times 10^{3}\pm 0.70$	$1.0^{c}\times 10^{4}\pm 3.53$
Escherichia coli	3	/	/	/
Enterobacteriaceae	3	/	/	/
30 danproizvodnja *30th day of the production*				
Ukupanbrojbakterija *Total bacteria count*	3	$4.3^{a}\times 10^{4}\pm 14.14$	$2.4^{b}\times 10^{4}\pm 7.07$	$3.8^{c}\times 10^{4}\pm 10.61$
Lactobacillus sp.	3	$2.5^{a}\times 10^{2}\pm 3.53$	$9.0^{b}\times 10^{2}\pm 10.60$	$4.0^{c}\times 10^{3}\pm 21.21$
Escherichia coli	3	/	/	/
Enterobacteriaceae	3	/	/	/

^{a,b,c}– the values marked with different letters have a statistically significant difference between the examined variants ($p<0.05$)

Moreover, the increase in the number of *Lactobacillus* sp. in variants 2 and 3 compared to the raw material is probably due to the initial action of the added starter cultures, and the minimal increase in the control variant is probably due to the development of the native lactobacilli microflora (Nieminen et al., 2011). The culmination of bacterial growth occurs in the initial stage of heat treatment of sausages (2 hours at a temperature of 42 °C). This is actually an incubation phase and is not usually used in routine industrial sausage production, but in the case of this study, the introduction of this phase was inevitable in order for the added starter cultures to become the dominant microflora and to achieve the desired effect in sausages.

According to the obtained results (Table 2), on the 10th day of production the highest total number of aerobic mesophilic bacteria (7.7×10^5 cfu/g) is determined

in the samples of sausages of variant 3, while the lowest in the sausages of the control variant (5.7×10^5 cfu/g).However, these values are lower than those in the filling, which is to be expected due to the heat treatment of the fresh sausages. On the 20th day of production, the total bacteria count is still decreasing. On the 30th day of production, the highest total bacteria count is determined in control variant ($4. \times 10^4$ cfu/g). In the samples from variant 3, the total bacteria count is$3.8 \cdot 10^4$ cfu/g. A statistically significant difference ($p<0.05$) in the total bacteria count on the 10th, 20th and 30th day of production is determined in the sausages of variants 2 and 3 compared to the control variant. On the other hand, the number of *Lactobacillus* sp. in the sausage samples in all three variants also decreases compared to the initial value. Thus, on the 10th day of production ($9.0 \cdot 10^4$ cfu/g), on the 20th day (1.0×10^4 cfu/g) and on the 30th day of production (4.0×10^3 cfu/g) the highest number of *Lactobacillus* sp. is determined in the samples of variant 3, compared to the samples from other two variants.This is due to the added starter cultures, i.e. the larger initial number of bacteria (Zdolec et al., 2007). Statistically significant difference ($p<0.05$) in the number of *Lactobacillus* sp. in the sausage samples on the 10th, 20th and 30th day of production was determined in variants 2 and 3 compared to the control variant. Moreover, on none of the examined days, in any of the variants there is no presence of *Escherichia coli* and *Enterobacteriaceae.*

According to Frece et al. (2010), by adding lactic acid bacteria during the production of traditional sausages, inhibition of undesirable microflora is achieved from 20 to 75%. The strongest inhibitory effect, according to the authors, was achieved on *Salmonella* sp. (75%) and *Escherichia coli* (73.80%), and slightly less on *Enterobacteriaceae*.Savić and Savić (2003), pointed out that the difference between industrially produced sausages and traditional sausages to which starter cultures have been added, in terms of microbiological quality is minimal, and they are most noticeable in the first days of production, where in sausages in which starter cultures were added, the number of lactic acid bacteria was higher, which results in greater stability of the product, as is the case in this study.

Conclusion

According to the obtained results, can be concluded that the sausages where starter cultures have been added are characterized with better microbiological stability compared to the sausages from the control variant. Moreover, there is no presence, neither of *Escherichia coli* nor of *Enterobacteriaceae*.From this point of view, starter cultures have a positive influence on the microbiological stability of the industrially produced *Macedonian traditional sausage*. Based on this research, starter culture CS-300 (*Staphylococcus carnosus* ssp. *utilis*) is recommended for future use. At the same time, the use of nitrite salt is eliminated, which results in getting a safe product.

References

Aymerich T., Martín B., Garriga M., Hugas, M. (2003).Microbial quality and direct PCR identification of lactic acid bacteria and nonpathogenic staphylococci from artisanal low-acid sausages, Applied and Environmental. Microbiology (69): 4583–4594.

Beuchat L.R., Montville T.J. (2001). Food microbiology: fundamentals and frontiers. 2nd ed. Washington (DC): American Society for Microbiology.

Chartier F.J.M., Couture M. (2004).Stability of the heme environment of the nitric oxide synthase from *Staphylococcus aureus* in the absence of pterin cofactor. Biophysical Journal (87): 1939–1950.

Fontana C., Cocconcelli S.P., Vignolo G. (2005).Monitoring the bacterial population dynamics during fermentation of artisanal Argentinean sausages.Int. J. Food Microbiol. (25): 131–42.

FreceJ.,Pleadin J., MarkovK., Perši H.,Dukić B.,Čvek D., Delaš F. (2010). Mikrobnapopulacija, kemijskisastav i mikotoksini u kobasicama s područjaVaraždinskežupanije.VeterinarnaStanica 41(3): 189-198.

Honikel K.O. (2008). The use and control of nitrate and nitrite for the processing of meat products. Meat Science 78, 1–2: 68–76.

ISO 7218:2007, Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations.

Janssens M., Myter N., De Vuyst L., Leroy F. (2012). Species diversity and metabolic impact of the microbiota are low in spontaneously acidified Belgian sausages with an added starter culture of *Staphylococcus carnosus*. Food Microbiol. 29: 167–177.

Kimiran Erdem A., Saglam D., Ozer D., Ozcelik E. (2014). Microbiological Quality of Minced Meat Samples Marketed in Istanbul. YYU VeterinerFakultesiDergisi, 25 (3): 67–70.

Nieminen T.T., Vihavainen E., Paloranta A., Lehto J., Paulin L., Auvinen P., Solismaa M., Björkroth K.J. (2011). Characterization of psychrotrophic bacterial communities in modified atmosphere-packed meat with terminal restriction fragment length polymorphism. Int J Food Microb 144: 360-366.

Savić Z., Savić I. (2003).Functionality of sausage casing, Victus, Wiena, pp. 41-93.

Vidal–Carou R. Talon R. (2007). Diversity of microorganisms in environments and dry fermented sausages of French traditional small units. Meat Science 76: 112–122.

Zara M.,Ionescu A.,Vasile A.,AproduI., Manoliu I. (2007). How starter cultures affect the sensorial characteristic and the microbiota evolution of dry sausages. Scientific Study & Research, ISSN 1582-540X, Vol. VIII (4): 379-390.

Zdolec N., Hadžiosmanović M., Kozačinski L., Cvrtila Ž., Filipović I., Leskovar K., Vragović N., Budimir D. (2007). Fermentiranekobasiceproizvedene u domaćinstvu - mikrobiološkakakvoća.Meso, Vol. IX studeni - prosinac br. 6: 318-324.

Ђукић Д., Мандић Л. (2012). Технологија микробиолошких производа. ИП „Просвета", Београд.

Ђукић Д., Мандић Л., Весковић С. (2015).Општа и индустријска микробиологија. Универзитет у Крагујевцу, Агрономски Факултет, Чачак.